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PATENT APPLICATION
Docket No. UMMC91-03A2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Harriet L. Robinson, Ellen F. Fynan, Robert G. Webster and Shan Lu

Serial No.: 08/187,879 Group Art Unit: 1804

Filed: January 27, 1994 Examiner: C. Hogue

Title: IMMUNIZATION BY INOCULATION OF DNA TRANSCRIPTION UNIT

Considered /DM/ 4/02

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Assistant Commissioner for Patents, Washington, D.C. 20231

on 2-28-96 Elizabeth M. Mahoney
Date Signature

Elizabeth M. Mahoney
Typed or printed name of person signing certificate

DECLARATION UNDER 37 C.F.R. 1.132 OF
DR. HARRIET L. ROBINSON

The Assistant Commissioner
for Patents
Washington, DC 20231

Sir:

I, Harriet L. Robinson, of 3 Birchwood Avenue, Southboro, Massachusetts 01772, hereby declare and state that:

1. I am a co-inventor on the above-identified patent application.

2. The following experiments were conducted by me or under my supervision.

3. A Simian Immunodeficiency Virus (SIV) DNA vaccine trial was conducted in monkeys. Six young adult female, and three young adult male Rhesus monkeys *(Macaca mulatta)*, which were negative for antibodies to SIV, simian retrovirus D, simian T-cell leukemia virus type 1, and Herpes simplex virus-1, were used. The protocol for the trial is similar to that described in the Specification at Example 14. The DNA transcription units used in the trial were similar to those described in the Specification at Example 13. Details of the exact protocol and DNA transcription units are described in Appendix A.

4. Rhesus macaques were inoculated with DNA transcription units at 1 and 3, 11 and 13, and 21 and 23 weeks. Four macaques were inoculated intravenously, intramuscularly and by gene gun inoculations ("multiple route" animals). Three received only gene gun inoculations, and two control monkeys were inoculated with control plasmids by all three routes of inoculation. The SIV challenge was administered intravenously two weeks following the last immunization. Antibody responses, complement-dependent antibody enhancement, susceptibility of challenge virus to raised antibody, CTL responses, post-challenge levels of infection, post-challenge CD4+ cell levels, and mortality were monitored.

5. The challenge infection raised high titers of neutralizing antibody for the challenge virus. Post-challenge titers of ELISA antibody for *Env* were approximately 100 times higher in the vaccinated groups than in the control group. Neutralizing antibody titers

were present in all DNA transcription unit-vaccinated monkeys after the second cluster of inoculations; these titers were transient, and were not boosted by the third cluster of inoculations. Complement-dependent enhancing antibodies could be detected in the sera of the vaccinated animals pre-challenge, and in the sera of both vaccinated and control animals post challenge. Cytotoxic T-cell activity for *Env* was also raised in all of the vaccinated animals. The temporal appearance of cytotoxic T-cells was similar to that of antibody. While antibody responses fell with time, cytotoxic T-cell responses persisted. The DNA immunizations did not prevent infection or protect against CD4+ cell loss. Long term chronic levels of infection were similar in the vaccinated and control animals. Notably, however, viral loads were reduced to the chronic level over a shorter period of time in the vaccinated animals (six weeks), than in the control animals (12 weeks).

6. The trial was terminated at one year post-challenge, and the animals were euthanized. At this time, the three animals in the "gene gun only" group, and one of the two control animals (the animal with the steady CD4+ levels), had succumbed to AIDS. The second control monkey and the four monkeys in the multiple route group did not have clinical signs of AIDS. The difference in survival did not correlate with differences in antibody and CTL responses, differences in levels of post-challenge infection, or differences in the rate of CD4+ cell decline.

I further declare that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be

true. Moreover, these statements were made with the knowledge that willful false statements and the like made by me are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Harriet L. Robinson

Harriet L. Robinson

2/22/96

Date